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(54) Title: COMBINATION THERAPY COMPRISING A DIARYL UREA COMPOUND AND A PI3, AKT KINASE OR MTOR INHIBITORS (RAPAMYCINS) FOR CANCER TREATMENT

(57) Abstract: The present invention relates to pharmaceutical compositions and combinations for treating cancer, comprising a diaryl urea compound e.g. 4 {4- [3- (4-chloro-3-trifluoromethylphenyl) -ureido] -3-fluorophenoxy}-pyridine-2- carboxyliσ acid methylamide and an PI3K/AKT signaling pathway inhibitor. The PI3K/AKI signaling pathway inhibitor comprises PI3 inhibitors {like celecoxilo, viridins, wortmannins} , AKT kinase inhibitors {like perifosine, triciribine} and mTOR inhibitors {like the rapamycins temsirolimus and evorolimus}.

COMBINATION THERAPY COMPRISING A DIARYL UREA COMPOUND AND A PI3, AKT KINASE OR MTOR INHIBITORS (RAPAMYCINS) FOR CANCER TREATMENT

BACKGROUND OF THE INVENTION

Diaryl urea compounds e.g. 4{4-[3-(4-chloro-3-trifluoromethylphenyl)-ureido]-3-fluorophenoxy}-pyridine-2-carboxylic acid methylamide as described e.g. in US 20050038080 are potent anti-cancer and anti-angiogenic agents that possess various activities, including inhibitory activity on the VEGFR, PDGFR, raf, p38, and/or flt-3 kinase signalling molecules. The RAS/RAF/MEK/ERK pathway is involved in cellular proliferation, differentiation, and transformation, and is implicated in many cancers. The PI3K/AKT signalling pathway is another important physiological pathway in cells. It mediates extracellular stimuli, including growth factors, cytokines, cell-cell adhesion and cell-extracellular matrices (Vivanco and Sawyers, Nat Rev Cancer, 2: 489-501, 2002, Downward, Curr Opin Cell Biol, 10: 262-267, 1998). The AKT pathway appears to be active in many types of human cancer (Nicholson and Anderson, Cell Signal, 14: 381-395, 2002).

DESCRIPTION OF THE INVENTION

The present invention provides drug combinations, compositions, and methods for treating diseases and conditions, including, but not limited to, cell proliferative disorders (such as cancer), inflammation, immunomodulatory disorders, and conditions associated with abnormal or undesirable angiogenesis. The drug combinations comprise a compound of formula I and at least one second compound that is an inhibitor of the PI3K/AKT signalling pathway. The methods can comprise, e.g., administering a diaryl urea compound as described below and a signalling pathway inhibitor, pharmaceutically-acceptable salts thereof, and derivatives thereof, etc.

The phosphatidylinositol-3-kinase (PI3K) and AKT (Protein Kinase B) signalling pathway regulates a variety of biological processes including cell survival, cell proliferation, cell growth, and cell motility. Abnormalities in PI3K-AKT signalling contribute to the pathogenesis of a number of diseases and conditions, including cell proliferative disorders (such as cancer), inflammation, and immunomodulatory disorders.

Many growth and survival factors activate PI3K family members to specifically convert one lipid signalling molecule, PIP2, into another, PI(3,4,5)P3. The phosphorylated product recruits Akt family members to the inner plasma membrane, stimulating their protein kinase activity. To date, many Akt effectors involved in several biological processes have been identified. For example, the Akt kinases mediate cell survival though phosphorylation and inactivation of apoptotic machinery components. The PI3K/AKT signalling pathway includes any members or components that

participate in the signal transduction cascade. These include, but are not limited to, e.g., PI3-kinase, Akt-kinase, FKBP12, mTOR (mammalian target of rapamycin; also known as FRAP, RAFT1, or RAPT1), RAPTOR (regulatory associated protein if mTOR), TSC (tuberous sclerosis complex), PTEN, (phosphatase and tensin homolog) and downstream effectors thereof. Combinations of the present invention can be used to treat and/or prevent any condition and/or diseases associated with any of the aforementioned activities.

An inhibitor of the PI3K/AKT signalling pathway is a compound that inhibits one or more members of the aforementioned signal transduction cascade. While such compounds may be referred to as pathway inhibitors, the present invention includes the use of these inhibitors to treat any of the mentioned diseases or conditions, regardless of the mechanism of action or how the therapeutic effect is achieved. Indeed, it is recognized that such compounds may have more than one target, and the initial activity recognized for a compound may not be the activity that it possesses *in vivo* when administered to a subject, or whereby it achieves its therapeutic efficacy. Thus, the description of a compound as a pathway or protein target (e.g., Akt or mTOR) inhibitor indicates that a compound possesses such activity, but in no way restricts a compound to having that activity when used as a therapeutic or prophylactic agent.

Examples of AKT family members include: Akt1, Akt2 (commonly over-expressed in tumors; Bellacosa et al., *Int. J. Cancer*, 64:280-285, 1995), and Akt3.

Examples of PI3K family members include: p110-alpha, p110-beta, p110-delta, and p110-gamma (catalytic).

Examples of PI3K/AKT signalling pathway inhibitors include, but are not limited to, e.g., FTY720 (e.g., Lee et al., *Carcinogenesis*, 25(12):2397-2405, 2004);

UCN-01 (e.g., Amornphimoltham et al., *Clin Cancer Res.*, 10(12 Pt 1):4029-37, 2004).

Examples of phosphatidylinositol-3-kinase (PI3-kinase) inhibitors, include, but are not limited to, e.g.,

celecoxib and analogs thereof, such as OSU-03012 and OSU-03013 (e.g., Zhu et al., Cancer Res., 64(12):4309-18, 2004);

3-deoxy-D-myo-inositol analogs (e.g., U.S. Application No. 20040192770; Meuillet et al., *Oncol. Res.*, 14:513-27, 2004), such as PX-316;

2'-substituted, 3'-deoxy-phosphatidyl-myo-inositol analogs (e.g., Tabellini et al., *Br. J. Haematol.*, 126(4):574-82, 2004);

fused heteroaryl derivatives (U.S. Pat. No. 6,608,056);

3-(imidazo[1,2-a]pyridin-3-yl) derivatives (e.g., U.S. Pat. Nos. 6,403,588 and 6,653,320);

Ly294002 (e.g., Vlahos, et al., *J. Biol., Chem.*, 269(7) 5241-5248, 1994);

quinazoline-4-one derivatives, such as IC486068 (e.g., U.S. Application No. 20020161014; Geng et al., *Cancer Res.*, 64:4893-99, 2004);

3-(hetero)aryloxy substituted benzo(b)thiophene derivatives (e.g., WO 04 108715; also WO 04 108713);

viridins, including semi-synthetic viridins such as such as PX-866 (acetic acid (1S,4E,10R,11R,13S,14R)-[4-diallylaminomethylene-6-hydroxy-1-methoxymethyl-10,13-dimethyl-3,7,17-trioxo-1,3,4,7,10,11,12,13,14,15,16,17-dodecahydro-2-oxa-cyclopenta[a]phenanthren-11-yl ester) (e.g., Ihle et al., *Mol Cancer Ther.*, 3(7):763-72, 2004; U.S. Application No. 20020037276; U.S. Pat. 5,726,167); and

wortmannin and derivatives thereof (e.g., U.S. Pat. Nos. 5,504,103; 5,480,906, 5,468,773; 5,441,947; 5,378,725; 3,668,222).

Examples of Akt-kinase (also known as protein kinase B) inhibitors, include, but are not limited to, e.g.,

Akt-1-1 (inhibits Akt1) (Barnett et al., *Biochem. J.*, 385 (Pt.2):399-408, 2005);

Akt-1-1,2 (inhibits Ak1 and 2) (Barnett et al., *Biochem. J.*, 385 (Pt.2):399-408, 2005);

API-59CJ-Ome (e.g., Jin et al., *Br. J. Cancer.*, 91:1808-12, 2004);

1-H-imidazo[4,5-c]pyridinyl compounds (e.g., WO05011700);

indole-3-carbinol and derivatives thereof (e.g., U.S. Pat. Nos. 6,656,963; Sarkar and Li, *J Nutr.*, 134(12 Suppl):3493S-3498S, 2004);

perifosine (e.g., interferes with Akt membrane localization; Dasmahapatra et al., *Clin. Cancer Res.*, 10(15):5242-52, 2004);

phosphatidylinositol ether lipid analogues (e.g., Gills and Dennis, *Expert. Opin. Investig. Drugs*, 13:787-97, 2004);

triciribine (TCN or API-2 or NCI identifier: NSC 154020; Yang et al., Cancer Res., 64:4394-9, 2004).

Examples of mTOR inhibitors include, but are not limited to, e.g.,

FKBP12 enhancer;

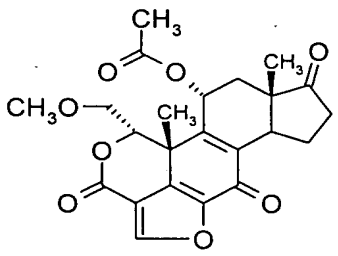
rapamycins and derivatives thereof, including: CCI-779 (temsirolimus), RAD001 (Everolimus; WO 9409010), TAFA93 and AP23573; rapalogs, e.g. as disclosed in WO 98/02441 and WO 01/14387, e.g. AP23573, AP23464, AP23675, or AP23841; 40-(2-hydroxyethyl)rapamycin, 40-[3-hydroxy(hydroxymethyl) methylpropanoate]-rapamycin (also called CC1779), 40-epi-(tetrazolyt)-rapamycin (also called ABT578), 32-deoxorapamycin, 16-pentynyloxy-32(S)-dihydrorapamycin, and other derivatives disclosed in WO 05005434; derivatives disclosed in USP 5,258,389, WO 94/090101, WO 92/05179, USP 5,118,677, USP 5,118,678, USP 5,100,883, USP 5,151,413, USP 5,120,842, WO 93/111130, WO 94/02136, WO 94/02485, WO 95/14023, WO 94/02136, WO 95/16691 (e.g. SAR 943), EP 509795, WO 96/41807, WO 96/41807 and USP 5,256, 790;

phosphorus-containing rapamycin derivatives (e.g., WO 05016252);

4H-1-benzopyran-4-one derivatives (e.g., U.S. Provisional Application No. 60/528,340).

Examples of compounds in preclinical or clinical use, include, e.g., AP23573, AP23841, CCI-779, and RAD001.

Examples of phosphatidylinositol-3-kinase (PI3-kinase) inhibitors of interest are wortmannin and the derivatives or analogs thereof and the pharmaceutically acceptable salts of wortmannin and its derivatives and analogs. Consequently, methods of this invention include the use of the PI3-kinase inhibitors of formula W:

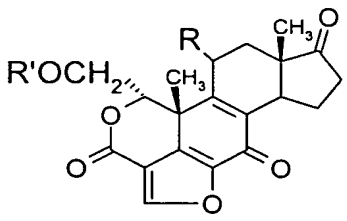


wortmannin
(W)

derivatives or analogs of the compound of formula W, pharmaceutically acceptable salts of the compound of formula W, and pharmaceutically acceptable salts of the derivatives or analogs of the compound of formula W.

Reference to the derivatives and analogs of wortmannin or the compound of "formula W" herein is intended to include the derivatives and analogs identified in U.S. Pat. Nos. 5,504,103; 5,480,906, 5,468,773; 5,441,947; 5,378,725; 3,668,222. Suitable derivatives and analogs of the compound of formula W include:

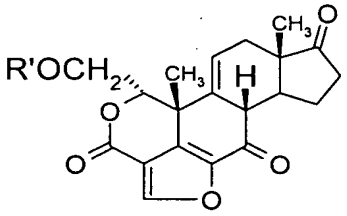
a) compounds of formula W1



W1

where R is H (11-desacetoxywortmannin) or acetoxy and R' is C_1-C_6 alkyl,

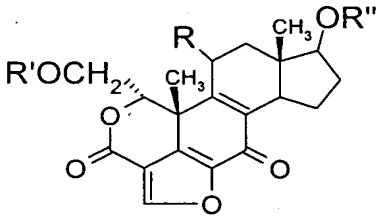
b) Δ9,11- dehydrodesacetoxywortmannin compounds of formula W2



W2

where R' is C_1-C_6 alkyl,

c) 17(α-dihydro-wortmannin compounds of formula W3

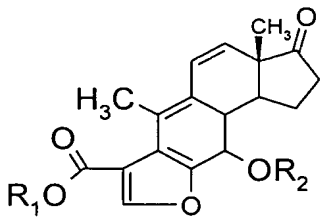


W3

where R is H or acetoxy and R' is C_1-C_6 alkyl, and R" is H, C_1-C_6 alkyl,

-C(O)OH or –C(O)O- C_1-C_6 alkyl;

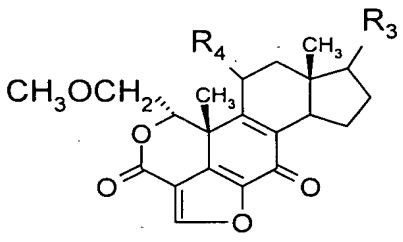
d) open A-ring acid or ester of wortmannin compounds of formula W4



W4

where R_1 is H, methyl or ethyl and R_2 is H or methyl or

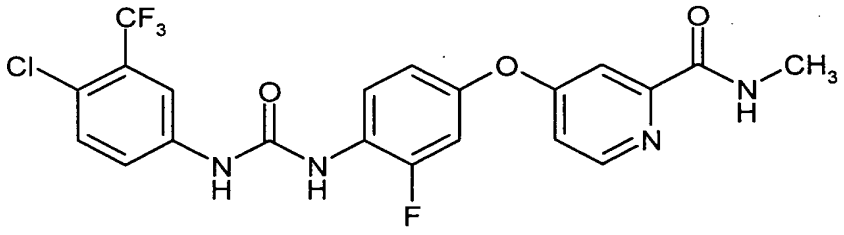
e) 11-substituted and 17- substituted derivatives of wortmannin of formula W5



W5

where R_4 is =O or –O(CO)R_6, R_3 is =O, -OH or –O(CO)R_6, each R_6 is independently phenyl, C_1-C_6 alkyl or substituted C_1-C_6 alkyl, where R_4 is =O or –OH, R_3 is not =O.

The compound with the structure of formula (I) wich corresponds to 4{4-[3-(4-chloro-3-trifluoromethylphenyl)-ureido]-3-fluorophenoxy}-pyridine-2-carboxylic acid methylamide, pharmaceutically acceptable salts, polymorphs, solvates, hydrates, metabolites and prodrugs thereof, are collectively referred to herein as the "compounds of formula I". formula (I) is as follows:



(I)

Where the plural form of the word compounds, salts, and the like, is used herein, this is taken to mean also a single compound, salt, or the like.

The term C_{1-6} alkyl, unless indicated otherwise, means straight, branched chain or cyclic alkyl groups having from one to six carbon atoms, which may be cyclic, linear or branched with single or multiple branching. Such groups include for example methyl, ethyl, *n*-propyl, isopropyl, *n*-butyl, isobutyl, *sec*-butyl, *tert*-butyl, cyclopropyl, cyclobutyl and the like. The present invention also relates to useful forms of the compounds as disclosed herein, such as pharmaceutically

acceptable salts and metabolites. The present invention also relatesto prodrugs of the compound of formula (I). The term "pharmaceutically acceptable salt" refers to a relatively non-toxic, inorganic or organic acid addition salt of a compound of the present invention. For example, see S. M. Berge, *et al.* "Pharmaceutical Salts," *J. Pharm. Sci.* 1977, 66, 1-19. Pharmaceutically acceptable salts include those obtained by reacting the main compound, functioning as a base, with an inorganic or organic acid to form a salt, for example, salts of hydrochloric acid, sulfuric acid, phosphoric acid, methane sulfonic acid, camphor sulfonic acid, oxalic acid, maleic acid, succinic acid and citric acid. Pharmaceutically acceptable salts also include those in which the main compound functions as an acid and is reacted with an appropriate base to form, e.g., sodium, potassium, calcium, mangnesium, ammonium, and choline salts. Those skilled in the art will further recognize that acid addition salts of the claimed compounds may be prepared by reaction of the compounds with the appropriate inorganic or organic acid via any of a number of known methods. Alternatively, alkali and alkaline earth metal salts are prepared by reacting the compounds of the invention with the appropriate base via a variety of known methods.

Representative salts of the compounds of this invention include the conventional non-toxic salts and the quaternary ammonium salts which are formed, for example, from inorganic or organic acids or bases by means well known in the art. For example, such acid addition salts include acetate, adipate, alginate, ascorbate, aspartate, benzoate, benzenesulfonate, bisulfate, butyrate, citrate, camphorate, camphorsulfonate, cinnamate, cyclopentanepropionate, digluconate, dodecylsulfate, ethanesulfonate, fumarate, glucoheptanoate, glycerophosphate, hemisulfate, heptanoate, hexanoate, hydrochloride, hydrobromide, hydroiodide, 2-hydroxyethanesulfonate, itaconate, lactate, maleate, mandelate, methanesulfonate, 2-naphthalenesulfonate, nicotinate, nitrate, oxalate, pamoate, pectinate, persulfate, 3-phenylpropionate, picrate, pivalate, propionate, succinate, sulfonate, tartrate, thiocyanate, tosylate, trifluoromethanesulfonate, and undecanoate.

Base salts include alkali metal salts such as potassium and sodium salts, alkaline earth metal salts such as calcium and magnesium salts, and ammonium salts with organic bases such as dicyclohexylamine and N-methyl-D-glucamine. Additionally, basic nitrogen containing groups may be quaternized with such agents as lower alkyl halides such as methyl, ethyl, propyl, and butyl chlorides, bromides and iodides; dialkyl sulfates like dimethyl, diethyl, and dibutyl sulfate; and diamyl sulfates, long chain halides such as decyl, lauryl, myristyl and strearyl chlorides, bromides and iodides, aryl or aralkyl halides like benzyl and phenethyl bromides and others monosubstituted aralkyl halides or polysubstituted aralkyl halides.

Solvates for the purposes of the invention are those forms of the compounds where solvent molecules form a complex in the solid state and include, but are not limited to for example ethanol

and methanol. Hydrates are a specific form of solvates, where the solvent molecule is water.

Certain pharmacologically active agents can be further modified with labile functional groups that are cleaved after *in vivo* administration to furnish the parent active agent and the pharmacologically inactive derivatizing group. These derivatives, commonly referred to as prodrugs, can be used, for example, to alter the physicochemical properties of the active agent, to target the active agent to a specific tissue, to alter the pharmacokinetic and pharmacodynamic properties of the active agent, and to reduce undesirable side effects. Prodrugs of the invention include, e.g., the esters of appropriate compounds of this invention that are well-tolerated, pharmaceutically acceptable esters such as alkyl esters including methyl, ethyl, propyl, isopropyl, butyl, isobutyl or pentyl esters. Additional esters such as phenyl-C_1-C_5 alkyl may be used, although methyl ester is preferred.

Methods which can be used to synthesize other prodrugs are described in the following reviews on the subject, which are incorporated herein by reference for their description of these synthesis methods:

- Higuchi, T.; Stella, V. eds. *Prodrugs As Novel Drug Delivery Systems.* ACS Symposium Series. American Chemical Society: Washington, DC (1975).
- Roche, E. B. *Design of Biopharmaceutical Properties through Prodrugs and Analogs.* American Pharmaceutical Association: Washington, DC (1977).
- Sinkula, A. A.; Yalkowsky, S. H. *J Pharm Sci.* **1975**, *64*, 181-210.
- Stella, V. J.; Charman, W. N. Naringrekar, V. H. *Drugs* **1985**, *29*, 455-473.
- Bundgaard, H., ed. *Design of Prodrugs.* Elsevier: New York (1985).
- Stella, V. J.; Himmelstein, K. J. *J. Med. Chem.* **1980**, *23*, 1275-1282.
- Han, H-K; Amidon, G. L. *AAPS Pharmsci* **2000**, *2*, 1- 11.
- Denny, W. A. *Eur. J. Med. Chem.* **2001**, *36*, 577-595.
- Wermuth, C. G. in Wermuth, C. G. ed. *The Practice of Medicinal Chemistry* Academic Press: San Diego (1996), 697-715.
- Balant, L. P.; Doelker, E. in Wolff, M. E. ed. *Burgers Medicinal Chemistry And Drug Discovery* John Wiley & Sons: New York (1997), 949-982.

The metabolites of the compounds of this invention include oxidized derivatives of the compounds of formula I, wherein one or more of the nitrogens are substituted with a hydroxy group; which includes derivatives where the nitrogen atom of the pyridine group is in the oxide form, referred to in the art as 1-oxo-pyridine or has a hydroxy substituent, referred to in the art as 1-hydroxy-pyridine.

General Preparative Methods

The compounds of the invention may be prepared by use of known chemical reactions and procedures as described e.g. in the following published international application WO 2005/009961.

The compounds of formula I have been previously characterized as having various activities, including for inhibiting the Raf/MEK/ERK pathway, raf kinase, p38 kinase, VEGFR kinase, PDGFR kinase. These activities and their use in treating various diseases and conditions are disclosed in, e.g., WO 2005/009961, which are hereby incorporated by reference in their entirety.

Indications

Drug combinations of the present invention can be utilized to treat any diseases or conditions that are associated with, or mediated by, the cellular pathways modulated by the compounds comprising the combinations. These pathways, include, but are not limited to signalling pathways which comprise, e.g., VEGFR, VEGFR2, Raf/Mek/Erk, Akt/PI3K, MTOR, PTEN, etc. (see also above). The drug combinations can be useful to treat diseases that are associated with, or mediated by, mutations in one of more genes present in these pathways, including cancer-associated mutations in PTEN, ras, Raf, Akt, PI3K, etc.

As mentioned above, although the compounds may be known as specific inhibitors, the present invention includes any ameliorative or therapeutic effect, regardless of the mechanism of action or how it is achieved.

The drug combination can have one or more of the following activities, including, anti-proliferative; anti-tumor; anti-angiogenic; inhibiting the proliferation of endothelial or tumor cells; anti-neoplastic; immunosuppressive; immunomodulatory; apoptosis-promoting, etc.

Conditions or diseases that can be treated in accordance with the present invention include proliferative disorders (such as cancer), inflammatory disorders, immuno-modulatory disorders, allergy, autoimmune diseases, (such as rheumatoid arthritis, or multiple sclerosis), abnormal or excessive angiogenesis, etc.

Any tumor or cancer can be treated, including, but not limited to, cancers having one or more mutations in raf, VEGFR-2, VEGFR-3, PDGFR-beta, Flt-3, ras, PTEN, Akt, PI3K, mTOR, as well as any upstream or downstream member of the signalling pathways of which they are a part. A tumor or cancer can be treated with a drug combination of the present invention irrespective of the mechanism that is responsible for it. Cancers of any organ can be treated, including cancers of, but are not limited to, e.g., colon, pancreas, breast, prostate, bone, liver, kidney, lung, testes, skin, pancreas, stomach, prostate, ovary, uterus, head and neck, blood cell, lymph, etc.

Cancers that can be treated in accordance with the present invention include, especially, but not limited to, brain tumors, breast cancer, bone sarcoma (e.g., osteosarcoma and Ewings sarcoma), bronchial premalignancy, endometrial cancer, glioblastoma, hematologic malignancies, hepatocellular carcinoma, Hodgkin's disease, kidney neoplasms, leukemia, leimyosarcoma, liposarcoma, lymphoma, Lhermitte-Duclose disease, malignant glioma, melanoma, malignant melanoma, metastases, multiple myeloma, myeloid metaplasia, myeloplastic syndromes, non-small cell lung cancer, pancreatic cancer, prostate cancer, renal cell carcinoma (e.g., advanced, advanced refractory), rhabdomyosarcoma, soft tissue sarcoma, squamous epithelial carcinoma of the skin, cancers associated with loss of function of PTEN; activated Akt (e.g. PTEN null tumors and tumors with ras mutations).

Examples of breast cancer include, but are not limited to, invasive ductal carcinoma, invasive lobular carcinoma, ductal carcinoma in situ, and lobular carcinoma in situ.

Examples of cancers of the respiratory tract include, but are not limited to, small-cell, non-small-cell lung carcinoma, bronchial adenoma, and pleuropulmonary blastoma.

Examples of brain cancers include, but are not limited to, brain stem and hypophtalmic glioma, cerebellar and cerebral astrocytoma, medulloblastoma, ependymoma, and neuroectodermal and pineal tumor.

Tumors of the male reproductive organs include, but are not limited to, prostate and testicular cancer. Tumors of the female reproductive organs include, but are not limited to, endometrial, cervical, ovarian, vaginal, and vulvar cancer, as well as sarcoma of the uterus.

Tumors of the digestive tract include, but are not limited to, anal, colon, colorectal, esophageal, gallbladder, gastric, pancreatic, rectal, small intestine, and salivary gland cancers.

Tumors of the urinary tract include, but are not limited to, bladder, penile, kidney, renal pelvis, ureter, and urethral cancers.

Eye cancers include, but are not limited to, intraocular melanoma and retinoblastoma.

Examples of liver cancers include, but are not limited to, hepatocellular carcinoma (liver cell carcinomas with or without fibrolamellar variant), cholangiocarcinoma (intrahepatic bile duct carcinoma), and mixed hepatocellular cholangiocarcinoma.

Skin cancers include, but are not limited to, squamous cell carcinoma, Kaposi's sarcoma, malignant melanoma, Merkel cell skin cancer, and non-melanoma skin cancer.

Head-and-neck cancers include, but are not limited to, laryngeal, hypopharyngeal, nasopharyngeal, and/or oropharyngeal cancers, and lip and oral cavity cancer.

Lymphomas include, but are not limited to, AIDS-related lymphoma, non-Hodgkin's lymphoma, cutaneous T-cell lymphoma, Hodgkin's disease, and lymphoma of the central nervous system.

Sarcomas include, but are not limited to, sarcoma of the soft tissue, osteosarcoma, malignant fibrous histiocytoma, lymphosarcoma, and rhabdomyosarcoma.

Leukemias include, but are not limited to, acute myeloid leukemia, acute lymphoblastic leukemia, chronic lymphocytic leukemia, chronic myelogenous leukemia, and hairy cell leukemia.

In addition to inhibiting the proliferation of tumor cells, drug combinations of the present invention can also cause tumor regression, e.g., a decrease in the size of a tumor, or in the extent of cancer in the body.

Preference is given to the treatment of melanoma, renal cancer, hepatocellular cancer, non small lung cancer, ovarian cancer, prostate cancer, colorectal cancer, breast cancer or pancreatic cancer.

Angiogenesis-related conditions and disorders can also be treated with drug combinations of the present invention. Inappropriate and ectopic expression of angiogenesis can be deleterious to an organism. A number of pathological conditions are associated with the growth of extraneous blood vessels. These include, e.g., diabetic retinopathy, neovascular glaucoma, psoriasis, retrolental fibroplasias, angiofibroma, inflammation, restenosis, etc. In addition, the increased blood supply associated with cancerous and neoplastic tissue, encourages growth, leading to rapid tumor enlargement and metastasis. Moreover, the growth of new blood vessels in a tumor provides an escape route for renegade cells, encouraging metastasis and the consequence spread of the cancer.

Useful systems for modulating angiogenesis, include, e.g., neovascularization of tumor explants (e.g., U.S. Pat. Nos. 5,192,744; 6,024,688), chicken chorioallantoic membrane (CAM) assay (e.g.,

Taylor and Folkman, Nature, 297:307-312, 1982; Eliceiri et al., J. Cell Biol., 140, 1255-1263, 1998), bovine capillary endothelial (BCE) cell assay (e.g., U.S. Pat. No. 6,024,688; Polverini, P. J. et al., Methods Enzymol., 198: 440-450, 1991), migration assays, and HUVEC (human umbilical cord vascular endothelial cell) growth inhibition assay (e.g., U.S. Pat. No. 6,060,449). In addition, useful systems for modulating lymphangiogenesis, include, e.g., rabbit ear model (e.g., Szuba et al., FASEB J., 16(14):1985-7, 2002).

Modulation of angiogenesis can be determined by any suitable method. For example, the degree of tissue vascularity is typically determined by assessing the number and density of vesssels present in a given sample. For example, microvessel density (MVD) can be estimated by counting the number of endothelial clusters in a high-power microscopic field, or detecting a marker specific for microvascular endothelium or other markers of growing or established blood vessels, such as CD31 (also known as platelet-endothelial cell adhesion molecule or PECAM). A CD31 antibody can be employed in conventional immunohistological methods to immunostain tissue sections as described by, e.g., Penfold et al., Br. J. Oral and Maxill. Surg., 34: 37-41; U.S. Pat. No. 6,017,949; Dellas et al., Gyn. Oncol., 67:27-33, 1997; and others. Other markers for angiogenesis, include, e.g., Vezf1 (e.g., Xiang et al., Dev. Bio., 206:123-141, 1999), angiopoietin, Tie-1, and Tie-2 (e.g., Sato et al., Nature, 376:70-74, 1995).

The drug combinations of this invention also have a broad therapeutic activity to treat or prevent the progression of a broad array of diseases, such as inflammatory conditions, coronary restenosis, tumor-associated angiogenesis, atherosclerosis, autoimmune diseases, inflammation, certain kidney diseases associated with proliferation of glomerular or mesangial cells, and ocular diseases associated with retinal vessel proliferation. psoriasis, hepatic cirrhosis, diabetes, atherosclerosis, restenosis, vascular graft restenosis, in-stent stenosis, angiogenesis, ocurlar diseases, pulmonary fibrosis, obliterative bronchiolitis, glomerular nephritis, rheumatoid arthritis.

The present invention also provides for treating, preventing, modulating, etc., one or more of the following conditions in humans and/or other mammals: retinopathy, including diabetic retinopathy, ischemic retinal-vein occlusion, retinopathy of prematurity and age related macular degeneration; rheumatoid arthritis, psoriasis, or bullous disorder associated with subepidermal blister formation, including bullous pemphigoid, erythema multiforme, or dermatitis herpetiformis, rheumatic fever, bone resorption, postmenopausal osteoperosis, sepsis, gram negative sepsis, septic shock, endotoxic shock, toxic shock syndrome, systemic inflammatory response syndrome, inflammatory bowel disease (Crohn's disease and ulcerative colitis), Jarisch-Herxheimer reaction, asthma, adult respiratory distress syndrome, acute pulmonary fibrotic disease, pulmonary sarcoidosis, allergic respiratory disease, silicosis, coal worker's pneumoconiosis, alveolar injury,

hepatic failure, liver disease during acute inflammation, severe alcoholic hepatitis, malaria (Plasmodium falciparum malaria and cerebral malaria), non-insulin-dependent diabetes mellitus (NIDDM), congestive heart failure, damage following heart disease, atherosclerosis, Alzheimer's disease, acute encephalitis, brain injury, multiple sclerosis (demyelation and oligiodendrocyte loss in multiple sclerosis), advanced cancer, lymphoid malignancy, pancreatitis, impaired wound healing in infection, inflammation and cancer, myelodysplastic syndromes, systemic lupus erythematosus, biliary cirrhosis, bowel necrosis, radiation injury/ toxicity following administration of monoclonal antibodies, host-versus-graft reaction (ischemia reperfusion injury and allograft rejections of kidney, liver, heart, and skin), lung allograft rejection (obliterative bronchitis), or complications due to total hip replacement, ad an infectious disease selected from tuberculosis, Helicobacter pylori infection during peptic ulcer disease, Chaga's disease resulting from Trypanosoma cruzi infection, effects of Shiga-like toxin resulting from E. coli infection, effects of enterotoxin A resulting from Staphylococcus infection, meningococcal infection, and infections from Borrelia burgdorferi, Treponema pallidum, cytomegalovirus, influenza virus, Theiler's encephalomyelitis virus, and the human immunodeficiency virus (HIV), papilloma, blastoglioma, Kaposi's sarcoma, melanoma, lung cancer, ovarian cancer, prostate cancer, squamous cell carcinoma, astrocytoma, head cancer, neck cancer, bladder cancer, breast cancer, colorectal cancer, thyroid cancer, pancreatic cancer, gastric cancer, hepatocellular carcinoma, leukemia, lymphoma, Hodgkin's disease, Burkitt's disease, arthritis, rheumatoid arthritis, diabetic retinopathy, angiogenesis, restenosis, in-stent restenosis, vascular graft restenosis, pulmonary fibrosis, hepatic cirrhosis, atherosclerosis, glomerulonophritis, diabetic nephropathy, thrombic micoangiopathy syndromes, transplant rejection, psoriasis, diabetes, wound healing, inflammation, and neurodegenerative diseases. hyperimmune disorders, hemangioma, myocardial angiogenesis, coronary and cerebral collateral vascularization, ischemia, corneal disease, rubeosis, neovascular glaucoma, macular degeneration retinopathy of prematurity, wound healing, ulcer Helicqbacter related diseases, fractures, endometriosis, a diabetic condition, cat scratch fever, thyroid hyperplasia, asthma or edema following burns, trauma, chronic lung disease, stroke, polyps, cysts, synovitis, chronic and allergic inflammation, ovarian hyperstimulation syndrome, pulmonary and cerebral edema, keloid, fibrosis, cirrhosis, carpal tunnel syndrome, adult respiratory distress syndrome, ascites, an ocular condition, a cardiovascular condition, Crow-Fukase (POEMS) disease, Crohn's disease, glomerulonophritis, osteoarthritis, multiple sclerosis, graft rejection, Lyme disease, sepsis, von Hippel Lindau disease, pemphigoid, Paget's disease, polycystic kidney disease, sarcoidosis, throiditis, hyperviscosity syndrome, Osler-Weber-Rendu disease, chronic occlusive pulmonary disease, radiation, hypoxia, preeclampsia, menometrorrhagia, endometriosis, infection by Herpes simplex, ischemic retinopathy, corneal angiogenisis, Herpes Zoster, human immunodeficiency virus, parapoxvirus, protozoa, toxoplasmosis, spondylarthritis, ankylosing

spondylitis, Morbus Bechterew, avian influenza including e.g. serotype H5N1, and tumor-associated effusions and edema.

The present invention provides methods of treating any of the aforementioned diseases and/or conditions (including those mentioned in any of the cited references), comprising administering effective amounts of a compound of formula I and at least one second compound that is an inhibitor of the PI3K/AKT signalling pathway (e.g. rapamycin or a derivative or analog of rapamycin, or wortmannin or a derivative or analog of wortmannin). An "effective amount" is the quantity of the compound that is useful to achieve the desired result, e.g., to treat the disease or condition.

The present invention also relates to methods of inhibiting angiogenesis in a system comprising cells, comprising administering to the system a combination of effective amounts of compounds described herein. A system comprising cells can be *an in vivo* system, such as a tumor in a patient, isolated organs, tissues, or cells, in vitro assays systems (CAM, BCE, etc), animal models (e.g., *in vivo*, subcutaneous, cancer models), hosts in need of treatment (e.g., hosts suffering from diseases having an angiogenic component, such as cancer; experiencing restenosis), etc.

In addition, the drug combinations can be administered to modulate one or more the following processes, cell growth (e.g., proliferation), tumor cell growth (including, e.g., differentiation, cell survival, and/or proliferation), tumor regression, endothelial cell growth (including, e.g., differentiation, cell survival, and/or proliferation), angiogenesis (blood vessel growth), angiogenesis, and/or hematopoiesis (e.g., proliferation, T-cell development, etc.).

Compounds or drug combinations of the present invention can be administered in any form by any effective route, including, e.g., oral, parenteral, enteral, intravenous, intraperitoneal, topical, transdermal (e.g., using any standard patch), ophthalmic, nasally, local, non-oral, such as aerosal, inhalation, subcutaneous, intramuscular, buccal, sublingual, rectal, vaginal, intra-arterial, and intrathecal, etc. They can be administered alone, or in combination with any ingredient(s), active or inactive. They can be administered in any effective dosage, e.g., from about 0.1 to about 200 mg/kg of total body weight.

The combinations of the present invention can be administered at any time and in any effective form. For example, the compounds can be administered simultaneously, e.g., as a single composition or dosage unit (e.g., a pill or liquid containing both compositions), or they can be administered as separate compositions, but at the same time (e.g., where one drug is administered intravenously and the other is administered orally or intramuscularly. The drugs can also be administered sequentially at different times. Agents can be formulated conventionally to achieve

the desired rates of release over extended period of times, e.g., 12-hours, 24-hours. This can be achieved by using agents and/or their derivatives which have suitable metabolic half-lives, and/or by using controlled release formulations.

The drug combinations can be synergistic, e.g., where the joint action of the drugs is such that the combined effect is greater than the algebraic sum of their individual effects. Thus, reduced amounts of the drugs can be administered, e.g., reducing toxicity or other deleterious or unwanted effects, and/or using the same amounts as used when the agents are administered alone, but achieving greater efficacy, e.g., in having more potent antiproliferative and pro-apoptotic action.

Compounds or drug combinations of the present invention can be further combined with any other suitable additive or pharmaceutically acceptable carrier. Such additives include any of the substances already mentioned, as well as any of those used conventionally, such as those described in Remington: The Science and Practice of Pharmacy (Gennaro and Gennaro, eds, 20th edition, Lippincott Williams & Wilkins, 2000); Theory and Practice of Industrial Pharmacy (Lachman et al., eds., 3rd edition, Lippincott Williams & Wilkins, 1986); Encyclopedia of Pharmaceutical Technology (Swarbrick and Boylan, eds., 2nd edition, Marcel Dekker, 2002). These can be referred to herein as "pharmaceutically acceptable carriers" to indicate they are combined with the active drug and can be administered safely to a subject for therapeutic purposes.

In addition, compounds or drug combinations of the present invention can be administered with other active agents or therapies (e.g., radiation) that are utilized to treat any of the above-mentioned diseases and/or conditions.

The present invention provides combinations of at least one compound of Formula I and at least one second compound which is a PI3K/AKT signalling pathway inhibitor useful in treating a disease or disorder. "Combinations" for the purposes of the invention include:

-single compositions or dosage forms which contain at least one compound of Formula I and at least one second compound which is an PI3K/AKT signalling pathway inhibitor;

-combination packs containing at least one compound of Formula I and at least one second compound which is an PI3K/AKT signalling pathway inhibitor, to be administered concurrently or sequentially;

-kits which comprise at least one compound of Formula I and at least one second compound which is an PI3K/AKT signalling pathway inhibitor packaged separate from one another as unit dosages or as independent unit dosages, with or without instructions that they be administered concurrently or sequentially; and

-separate independent dosage forms of at least one compound of Formula I and at least one second compound which is an PI3K/AKT signalling pathway inhibitor which cooperate to achieve a therapeutic effect, e.g., prophylaxis or treatment of the same disease, when administered concurrently or sequentially.

The dosage of each agent of the combination can be selected with reference to the other and/or the type of disease and/or the disease status in order to provide the desired therapeutic activity. For example, the active agents in the combination can be present and administered in a fixed combination. "Fixed combination" is intended here to mean pharmaceutical forms in which the components are present in a fixed ratio that provides the desired efficacy. These amounts can be determined routinely for a particular patient, where various parameters are utilized to select the appropriate dosage (e.g., type of cancer, age of patient, disease status, patient health, weight, etc.), or the amounts can be relatively standard.

The combination can comprise effective amounts of at least one compound of Formula I and at least one second compound which is a PI3K/AKT signalling pathway inhibitor, which achieves a greater therapeutic efficacy than when either compound is used alone. The combination can be useful to produce tumor regression, to produce disease stability, to prevent or reduce metastasis, or other therapeutic endpoints, where the therapeutic effect is not observed when the agents are used alone, or where an enhanced effect is observed when the combination is administered.

The relative ratios of each compound in the combination can also be selected based on their respective mechanisms of action and the disease biology. For example, activating mutations of the B-RAF gene are observed in more than 60% of human melanomas and a composition for treatment of melanoma may advantageously comprise a formula I compound in a more potent amount than the compound which is a P13K/AKT signalling pathway inhibitor. In comparison, where a cancer is associated with a mutation in the PI3K/AKT signalling pathway (e.g., ovarian and breast cancers), an agent which has activity in this signalling pathway can be present in more potent amounts relative to the Ref/MEK/ERK pathway inhibitor. The relative ratios of each compound can vary widely and this invention includes combinations for treating cancer where the amounts of the formula I compound and the second active agent can be adjusted routinely such that either is present in higher amounts.

The release of one or more agents of the combination can also be controlled, where appropriate, to provide the desired therapeutic activity when in a single dosage form, combination pack, kit or when in separate independent dosage forms.

Assays

Activity of combinations of the present invention can be determined according to any effective *in vitro* or *in vivo* method.

Kinase activity

Kinase activity can be determined routinely using conventional assay methods. Kinase assays typically comprise the kinase enzyme, substrates, buffers, and components of a detection system. A typical kinase assay involves the reaction of a protein kinase with a peptide substrate and an ATP, such as ^{32}P-ATP, to produce a phosphorylated end-product (for instance, a phosphoprotein when a peptide substrate is used). The resulting end-product can be detected using any suitable method. When radioactive ATP is utilized, a radioactively labeled phosphoprotein can be separated from the unreacted gamma-^{32}P-ATP using an affinity membrane or gel electrophoresis, and then visualized on the gel using autoradiography or detected with a scintillation counter. Non-radioactive methods can also be used. Methods can utilize an antibody which recognizes the phosphorylated substrate, e.g., an anti-phosphotyrosine antibody. For instance, kinase enzyme can be incubated with a substrate in the presence of ATP and kinase buffer under conditions which are effective for the enzyme to phosphorylate the substrate. The reaction mixture can be separated, e.g., electrophoretically, and then phosphorylation of the substrate can be measured, e.g., by Western blotting using an anti-phosphotyrosine antibody. The antibody can be labeled with a detectable label, e.g., an enzyme, such as HRP, avidin or biotin, chemiluminescent reagents, etc. Other methods can utilize ELISA formats, affinity membrane separation, fluorescence polarization assays, luminescent assays, etc.

An alternative to a radioactive format is time-resolved fluorescence resonance energy transfer (TR-FRET). This method follows the standard kinase reaction, where a substrate, e.g., biotinylated poly(GluTyr), is phosphorylated by a protein kinase in the presence of ATP. The end-product can then detected with a europium chelate phosphospecific antibody (anti-phosphotyrosine or phosphoserine/threonine), and streptavidin-APC, which binds the biotinylated substrate. These two components are brought together spatially upon binding, and energy transfer from the phosphospecific antibody to the acceptor (SA-APC) produces fluorescent readout in the homogeneous format.

Raf/MEK/ERK activity

A c-Raf kinase assay can be performed with a c-Raf enzyme activated (phosphorylated) by Lck kinase. Lck-activated c-Raf (Lck/c-Raf) is produced in Sf9 insect cells by co-infecting cells with baculoviruses expressing, under the control of the polyhedrin promoter, GST-c-Raf (from amino acid 302 to amino acid 648) and Lck (full-length). Both baculoviruses are used at the multiplicity of infection of 2.5 and the cells are harvested 48 hours post infection.

MEK-1 protein is produced in Sf9 insect cells by infecting cells with the baculovirus expressing GST-MEK-1 (full-length) fusion protein at the multiplicity of infection of 5 and harvesting the cells 48 hours post infection. Similar purification procedure is used for GST-c-Raf 302-648 and GST-MEK-1.

Transfected cells are suspended at 100 mg of wet cell biomass per mL in a buffer containing 10 mM sodium phosphate, 140 mM sodium chloride pH 7.3, 0.5% Triton X-100 and the protease inhibitor cocktail. The cells are disrupted with a Polytron homogenizer and centrifuged 30,000g for 30 minutes. The 30,000g supernatant is applied applied onto GSH-Sepharose. The resin is washed with a buffer containing 50 mM Tris, pH 8.0, 150 mM NaCl and 0.01% Triton X-100. The GST-tagged proteins are eluted with a solution containing 100 mM Glutathione, 50 mM Tris, pH 8.0, 150 mM NaCl and 0.01% Triton X-100. The purified proteins are dialyzed into a buffer containing 20 mM Tris, pH 7.5, 150 mM NaCl and 20% Glycerol.

Test compounds are serially diluted in DMSO using three-fold dilutions to stock concentrations ranging typically from 50 μM to 20 nM (e.g., final concentrations in the assay can range from 1 μM to 0.4 nM). The c-Raf biochemical assay is performed as a radioactive filtermat assay in 96-well Costar polypropylene plates (Costar 3365). The plates are loaded with 75 μL solution containing 50 mM HEPES pH 7.5, 70 mM NaCl, 80 ng of Lck/c-Raf and 1 μg MEK-1. Subsequently, 2 μL of the serially diluted individual compounds is added to the reaction, prior to the addition of ATP. The reaction is initiated with 25 μL ATP solution containing 5μM ATP and 0.3 μCi [33P]-ATP. The plates were sealed and incubated at 32°C for 1 hour. The reaction is quenched with the addition of 50 μl of 4 % Phosphoric Acid and harvested onto P30 filtermats (PerkinElmer) using a Wallac Tomtec Harvester. Filtermats are washed with 1 % Phosphoric Acid first and deinonized H2O second. The filters are dried in a microwave, soaked in scintillation fluid and read in a Wallac 1205 Betaplate Counter (Wallac Inc., Atlanta, GA, U.S.A.). The results are expressed as percent inhibition.

% Inhibition = [100-(Tib/Ti)] x 100 where

Tib = (counts per minute with inhibitor)-(background)

Ti = (counts per minute without inhibitor)-(background)

Raf activity can also be monitored by its ability to initiate the cascade leading to ERK phosphorylation (i.e., raf/MEK/ERK), resulting in phospho-ERK. A Bio-Plex Phospho-ERK1/2 immunoassay can be performed as follows:

A 96-well phospho-ERK (pERK) immunoassay, using laser flow cytometry platform has been established to measure inhibition of basal pERK in cell lines. MDA-MB-231 cells are plated at 50,000 cells per well in 96-well microtitre plates in complete growth media. For effects of test compounds on basal pERK1/2 inhibition, the next day after plating, MDA-MB-231 cells are transferred to DMEM with 0.1% BSA and incubated with test compounds diluted 1:3 to a final concentration of 3 mM to 12 nM in 0.1% DMSO. Cells are incubated with test compounds for 2 h, washed, and lysed in Bio-Plex whole cell lysis buffer A. Samples are diluted with buffer B 1:1 (v/v) and directly transferred to assay plate or frozen at –80 C degrees until processed. 50 mL of diluted MDA-MB-231 cell lysates are incubated with about 2000 of 5 micron Bio-Plex beads conjugated with an anti-ERK1/2 antibody overnight on a shaker at room temperature. The next day, biotinylated phospho-ERK1/2 sandwich immunoassay is performed, beads are washed 3 times during each incubation and then 50 mL of PE-strepavidin is used as a developing reagent. The relative fluorescence units of pERK1/2 is detected by counting 25 beads with Bio-Plex flow cell (probe) at high sensitivity. The IC50 is calculated by taking untreated cells as maximum and no cells (beads only) as background.

Phosphatidylinositol 3-kinase activity

PKI3 activity can be determined routinely, e.g., using commercially available kits (e.g., Perkin-Elmer, FlashPlate Platform), Frew et al., *Anticancer Res.*, 14(6B):2425-8, 1994. See also, publications listed under PKI3 inhibitors.

Akt activity

AKT can be isolated from insect cells expressing His-tagged AKT1 (aa 136-480) as described in WO 05011700. Expressing cells are lysed in 25 mM HEPES, 100 mM NaCl, 20 mM imidazole; pH 7.5 using a polytron (5 mls lysis buffer/g cells). Cell debris is removed by centrifuging at 28,000 x g for 30 minutes. The supernatant is filtered through a 4.5 micron filter then loaded onto a nickel-chelating column pre-equilibrated with lysis buffer. The column is washed with 5 column volumes (CV) of lysis buffer then with 5 CV of 20% buffer B, where buffer B is 25 mM HEPES, 100 mM NaCl, 300 mM imidazole; pH 7. His-tagged AKT1 (aa 136-480) is eluted with a 20-100%

linear gradient of buffer B over 10 CV. His-tagged AKTI (136-480) eluting fractions are pooled and diluted three-fold with buffer C, where buffer C is 25 mM HEPES, pH 7. The sample is then chromatographed over a Q-Sepharose HP column pre-equilibrated with buffer C. The column is washed with 5 CV buffer C, then step eluted with 5 CV 10 %D, 5 CV 20%D, 5 CV 30% D, 5 CV 50% D. and 5 CV of 100% D; where buffer D is 25 mM HEPES, 1000 mM NaCl; pH 7.5.

His-tagged AKTI (aa 136-480) containing fractions are pooled and concentrated in a 10-kDa molecular weight cutoff concentrator. His-tagged AKT1 (aa 136-480) is chromatographed over a Superdex 75 gel filtration column pre-equilibrated with 25 mM HEPES, 200 mM NaCl, 1 mM DTT; pH 7.5. His-tagged AKT1 (aa 136-480) fractions are examined using SDS-PAGE and mass spec. The protein is pooled, concentrated, and stored at 80°C.

His-tagged AKT2 (aa 138-481) and His-tagged AKT3 (aa 135-479) can be isolated and purified in a similar fashion.

AKT Enzyme Assay Compounds can be tested for AKT protein serine kinase inhibitory activity in substrate phosphorylation assays. This assay examines the ability of small molecule organic compounds to inhibit the serine phosphorylation of a peptide substrate. The substrate phosphorylation assays use the catalytic domains of AKT 1, 2, or 3. AKT 17 2 and 3 are also commercially available from Upstate USA, Inc. The method measures the ability of the isolated enzyme to catalyze the transfer of the gamma-phosphate from ATP onto the serine - 72 residue of a biotinylated synthetic peptide (Biotin-ahx-ARKRERAYSFGHHA-amide). Substrate phosphorylation can be detected by the following procedure described in WO 05011700.

Assays are performed in 384 well U-bottom white plates. 10 nM activated AKT enzyme is incubated for 40 minutes at room temperature in an assay volume of 20ul containing 5O mM MOPS, pH 7.5, 20 mM $MgCl_2$, 4uM ATP, 8uM peptide, 0.04 uCi [g- ^{33}P] ATP/well, 1 mM CHAPS, 2 mM DTT, and 1 µl of test compound in 100% DMSO. The reaction is stopped by the addition of 50 µl SPA bead mix (Dulbecco's PBS without Mg2+ and Ca2+, 0.1 % Triton X-100, 5 mM EDTA, 50 µM ATP, 2.5mg/ml Streptavidin-coated SPA beads). The plate is sealed, the beads are allowed to settle overnight, and then the plate was counted in a Packard Topcount Microplate Scintillation Counter (Packard Instrument Co., Meriden, CT).

The data for dose responses can be plotted as % Control calculated with the data reduction formula 100*(U1-C2)/(C1-C2) versus concentration of compound where U is the unknown value, C1 is the average control value obtained for DIVISO, and C2 is the average control value obtained for 0.1M EDTA. Data are fitted to the curve described by: y = ((Vmax * x) K + x)) where Vmax is the upper asymptote and K is the IC50.

Cell proliferation

An example of a cell proliferation assay is described in the Examples below. However, proliferation assays can be performed by any suitable method. For example, a breast carcinoma cell proliferation assay can be performed as follows. Other cell types can be substituted for the MDA-MB-231 cell line.

Human breast carcinoma cells (MDA MB-231, NCI) are cultured in standard growth medium (DMEM) supplemented with 10% heat-inactivated FBS at 37°C in 5% CO_2 (vol/ vol) in a humidified incubator. Cells are plated at a density of 3000 cells per well in 90 µL growth medium in a 96 well culture dish. In order to determine T_{0h} CTG values, 24 hours after plating, 100 µL of CellTiter-Glo Luminescent Reagent (Promega) is added to each well and incubated at room temperature for 30 minutes. Luminescence is recorded on a Wallac Victor II instrument. The CellTiter-Glo reagent results in cell lysis and generation of a luminescent signal proportional to the amount of ATP present, which, in turn is directly proportional to the number of cells present.

Test compounds are dissolved in 100% DMSO to prepare 10 mM stocks. Stocks are further diluted 1:400 in growth medium to yield working stocks of 25 µM test compound in 0.25% DMSO. Test compounds are serially diluted in growth medium containing 0.25% DMSO to maintain constant DMSO concentrations for all wells. 60 µL of diluted test compound are added to each culture well to give a final volume of 180 µL. The cells with and without individual test compounds are incubated for 72 hours at which time ATP dependent luminescence was measured, as described previously, to yield T_{72h} values. Optionally, the IC_{50} values can be determined with a least squares analysis program using compound concentration versus percent inhibition.

$$\% \text{ Inhibition} = [1-(T_{72h\ test}-T_{0h})/(T_{72h\ ctrl}-T_{0h})] \times 100, \text{ where}$$

$T_{72h\ test}$ = ATP dependent luminescence at 72 hours in the presence of test compound

$T_{72h\ ctrl}$ = ATP dependent luminescence at 72 hours in the absence of test compound

T_{0h} = ATP dependent luminescence at Time Zero.

Angiogenesis

One useful model to study angiogenesis is based on the observation that, when a reconstituted basement membrane matrix, such as Matrigel, supplemented with growth factor (e.g., FGF-1), is injected subcutaneously into a host animal, endothelial cells are recruited into the matrix, forming new blood vessels over a period of several days. See, e.g., Passaniti et al., *Lab. Invest.*, 67:519-

528, 1992. By sampling the extract at different times, angiogenesis can be temporally dissected, permitting the identification of genes involved in all stages of angiogenesis, including, e.g., migration of endothelial cells into the matrix, commitment of endothelial cells to angiogenesis pathway, cell elongation and formation of sac-like spaces, and establishment of functional capillaries comprising connected, and linear structures containing red blood cells. To stabilize the growth factor and/or slow its release from the matrix, the growth factor can be bound to heparin or another stabilizing agent. The matrix can also be periodically re-infused with growth factor to enhance and extend the angiogenic process.

Other useful systems for studying angiogenesis, include, e.g., neovascularization of tumor explants (e.g., U.S. Pat. Nos. 5,192,744; 6,024,688), chicken chorioallantoic membrane (CAM) assay (e.g., Taylor and Folkman, Nature, 297:307-312, 1982; Eliceiri et al., J. Cell Biol., 140, 1255-1263, 1998), bovine capillary endothelial (BCE) cell assay (e.g., U.S. Pat. No. 6,024,688; Polverini, P. J. et al., Methods Enzymol., 198: 440-450, 1991), migration assays, HUVEC (human umbilical cord vascular endothelial cell) growth inhibition assay (e.g., U.S. Pat. No. 6,060,449).

The present invention provides one or more of the following features.

A method of treating any of the aforementioned diseases and/or conditions, comprising administering effective amounts of a compound of formula I and a second compound which is an PI3K/AKT signalling pathway inhibitor.

A method of modulating (e.g., inhibiting) one or more aforementioned activities, comprising administering effective amounts of a compound of formula I and a second compound which is an PI3K/AKT signalling pathway inhibitor.

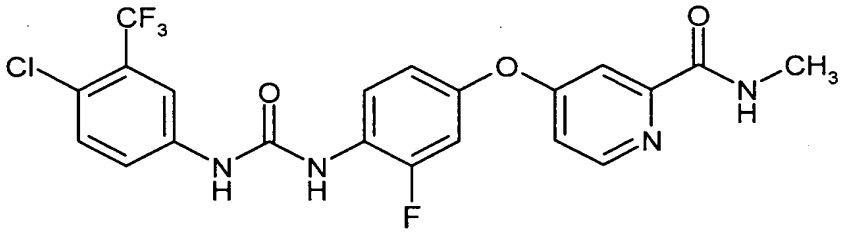
Combinations comprising a compound of formula I and a second compound which is an PI3K/AKT signalling pathway inhibitor.

Without further elaboration, it is believed that one skilled in the art can, using the preceding description, utilize the present invention to its fullest extent. The following preferred specific embodiments are, therefore, to be construed as merely illustrative, and not limitative of the remainder of the disclosure in any way whatsoever. The entire disclosure of all patents and publications, cited above and below are hereby incorporated by reference in their entirety.

What we claim:

1. A combination comprising

a compound of formula I



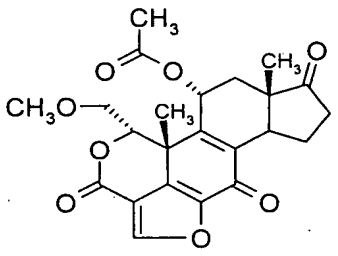
(I)

or a pharmaceutically acceptable salt, polymorph, solvate, hydrate, metabolite or prodrug form thereof, and at least one second compound which is an PI3K/AKT signalling pathway inhibitor.

2. A combination of claim 1, wherein said second compound is celecoxib, OSU-03012, OSU-03013, PX-316, 2'-substituted, 3'-deoxy-phosphatidyl-myo-inositol derivatives, 3-(imidazo[1,2-a]pyridin-3-yl) derivatives, Ly294002, IC486068, 3-(hetero)aryloxy substituted benzo(b)thiophene derivatives, PX-866, perifosine, triciribine, FKBP12 enhancer, phosphatidylinositol ether lipid analogues, wortmannin or rapamycin or derivatives thereof, or a pharmaceutically-acceptable salt thereof.

3. A combination of claim 1, wherein said second compound is a wortmannin compound of

formula W:



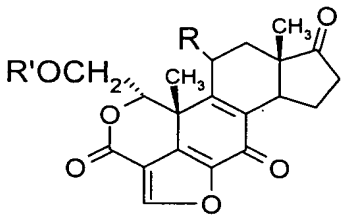
(W)

a derivative or analog of a wortmannin compound of formula W, a pharmaceutically acceptable salt of the wortmannin compound of formula W, or a pharmaceutically acceptable salt of the derivative or analog of the wortmannin compound of formula W.

4. A combination of claim 3, wherein said derivative or analog of the formula W

is selected from

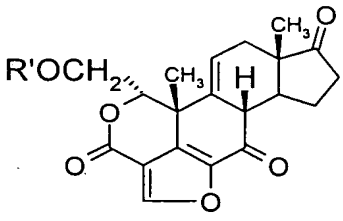
a) compounds of formula W1



W1

where R is H (11-desacetoxywortmannin) or acetoxy and R' is C_1-C_6alkyl,

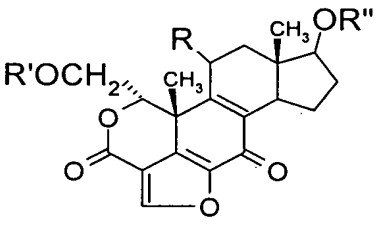
b) Δ9, 11- dehydrodesacetoxywortmannin compounds of formula W2



W2

where R' is C_1-C_6 alkyl,

c) 17(α-dihydro-wortmannin compounds of formula W3

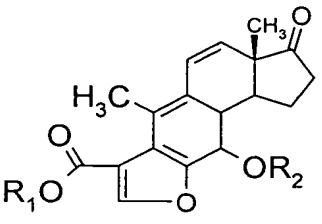


W3

where R is H or acetoxy and R' is C_1-C_6 alkyl and R" is H, C_1-C_6 alkyl,

-C (O) OH or –C (O) O- C_1-C_6 alkyl;

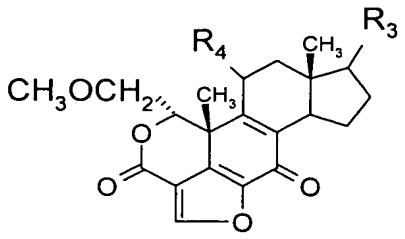
d) open A-ring acid or ester of wortmannin compounds of formula W4



W4

where R_1 is H, methyl or ethyl and R_2 is H or methyl or

e) 11-substituted and 17- substituted derivatives of wortmannin of formula W5



W5

where R_4 is =O or –O(CO)R_6, R_3 is =O, -OH or –O(CO)R_6, each R_6 is independently phenyl, C_1-C_6 alkyl or substituted C_1-C_6 alkyl, where R_4 is =O or –OH, R_3 is not =O.

5. A combination of claim 1, wherein said second compound is an Akt-kinase inhibitor.

6. A combination of claim 1, wherein said second compound is Akt-1-1, Akt-1-1,2, API-59CJ-Ome, 1-H-imidazo[4,5-c]pyridinyl derivatives, indole-3-carbinol and derivatives thereof, perifosine, phosphatidylinositol ether lipid analogues, triciribine, or a pharmaceutically-acceptable salt thereof.

7. A combination of claim 1, wherein said second compound is an mTOR inhibitor.

8. A combination of claim 1, wherein said second compound is rapamycin, temsirolimus, everolimus, AP23573, AP23675, AP23464, AP23841, 40-(2-hydroxyethyl)rapamycin, 40-[3-hydroxy(hydroxymethyl) methylpropanoate]-rapamycin, 40-epi-(tetrazolyt)-rapamycin, 32-deoxorapamycin, or 16-pentynyloxy-32(S)-dihydrorapamycin, SAR 943 or a pharmaceutically-acceptable salt thereof.

9. A combination of claim 1 comprising a compound of formula (I) and wortmannin.

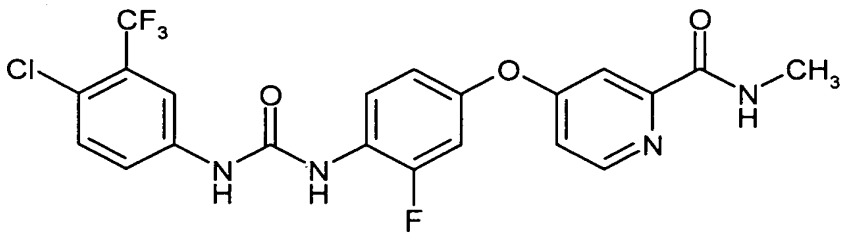
10. A combination of claim 1 comprising a compound of formula (I) and rapamycin.

11. A combination of any of claims 1 to 10 wherein the amounts of the active ingredients of the combination are synergistic.

12. A combination of any of claims 1 to 11 for treating cancer.

13. A combination of claim 12, wherein said cancer is melanoma, hepatocellular cancer, renal cell carcinoma non small lung cancer, ovarian cancer, prostate cancer, colorectal cancer, breast cancer or pancreatic cancer.

14. A method for treating cancer in a subject in need thereof comprising administering effective amounts of a compound of formula I



(I)

or a pharmaceutically acceptable salt, polymorph, solvate, hydrate, metabolite or prodrug thereof, and of a second compound which is an PI3K/AKT signalling pathway inhibitor.

15. Process for manufacturing of a combination of any of claims 1 to 11 for treating cancer.

16. Process of claim 15, wherein said cancer is melanoma, hepatocellular cancer, renal cell carcinoma, non small lung cancer, ovarian cancer, prostate cancer, colorectal cancer, breast cancer or pancreatic cancer.

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2006/004524

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K31/4412 A61K31/436 A61P35/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, PAJ, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2005/038080 A1 (BOYER STEPHEN ET AL) 17 February 2005 (2005-02-17) page 18 r-h col. example 1 page 17 [0217]	1-15
X	WO 2005/009961 A (BAYER PHARMACEUTICALS CORPORATION; DUMAS, JACQUES; BOYER, STEPHEN; RIE) 3 February 2005 (2005-02-03) page 44, last paragraph claim 1	1-16

[ ] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
20 July 2006	04/08/2006

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040, Tx. 31 651 epo nl, Fax: (+31–70) 340–3016	Baumgärtner, H

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP2006/004524

Box II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claim 14 is directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2006/004524

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 2005038080 A1	17-02-2005	NONE	
WO 2005009961 A	03-02-2005	AR 048741 A1	24-05-2006
		AU 2004259760 A1	03-02-2005
		CA 2532865 A1	03-02-2005
		EP 1663978 A2	07-06-2006
		HR 20060073 A2	30-06-2006